Microsatellite instability (MSI) is caused by deficient DNA mismatch repair, and results in a “mutator” phenotype. Recent studies have produced contradictory results about the frequency and significance of MSI in malignant melanomas. In this study, we therefore determined the time of onset and relative frequency of MSI during the progression of melanocytic tumours, including benign melanocytic naevi. We examined 7 different microsatellite loci in 9 melanocytic naevi, 25 primary malignant melanomas and 8 melanoma metastases. None of the melanocytic naevi showed MSI. In contrast, moderate frequency of MSI in 1/12 (8%) was detected in thin melanomas of < 0.75 mm vertical thickness and in 1/8 (12%) of those with a thickness > 0.75 mm and < 1.5 mm. The rate of MSI was increased in tumours thicker than 1.5 mm (2/5) and in melanoma metastases, with over 25% (2/8) of the lesions investigated. We conclude that MSI occurs in a considerable subset of malignant melanomas and that there is a pattern consistent with increasing frequency of MSI with progression of melanocytic tumours. Key words: malignant melanoma; microsatellite instability.

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Microsatellite Instability in Malignant Melanomas

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ACT CAC TCT AGT GAT AAA TCG, antisense: AGC AGA TAA GAC AGT ATT ACT AGT T; D10S89 sense: AAC ACT AGT GAC ATT ATT TTC, antisense: AGC TAG GCC TGA AGG CCT CT; D17S520 sense: AGG GAT ACT ATT CAG CCC GAG GTG, antisense: ACT GCC ACT CCT TGC CCC ATT C; D10S197 sense: ACC ACT GCA CCT CAG GTG AC, antisense: GTG ATA CTG TCC TCA GTA GTC C; D11S904 sense: ATG ACA AGC AAT CCT TGA GC, antisense: CTG TGT TAT ATC CCT AAA GTG GTG A; D13S175 sense: TAT TGG ATA CTT GAA TCT GCT G, antisense: TGC ACC TCA CAT AGG TTA; D9S171 sense: AGC TAA GTG AAC CTC TAC TTC GTC; D13S175 sense: TAT TGG ATA CTT GAA TCT GCT G, antisense: TGC ACC TCA CAT AGG TTA; D9S171 sense: AGC TAA GTG AAC CTC TAC TTC GTC.

For each pair of tumours and normal DNA, separate reactions with the above-mentioned set of primers were performed. For PCR 50 ng of each template DNA were added to a mixture of 1 × Taq buffer with 1.5 mM MgCl₂ (Boehringer, Mannheim, Germany), 200 mM dNTPs, 0.5 μM of each primer, and 5U/Rx Taq-Polymerase (Boehringer, Mannheim, Germany) in a volume of 50 μl. PCR conditions were 1 min at 95°C, 1 min at 55–60°C (depending on the primers) and 1 min 30s at 72°C. The PCR reaction products were then electrophoresed on 6% denaturing polyacrylamide gels and stained in accordance with a previously developed silver-staining protocol (19). MSI was assumed if at least one out of 7 of the tested loci showed somatic variations in microsatellite length. Loss of heterozygosity (LOH) was defined if in an informative microsatellite locus (showing both alleles) one allele was lost in the tumour sample and a shift of one allele to the length of the other allele could be excluded because of the equal intensity of the band.

RESULTS

The analysis of 9 benign melanocytic naevi, 24 sporadic primary cutaneous melanomas, and 8 metastases of malignant melanomas matched with normal tissue samples of each patient revealed MSI exclusively in malignant lesions (Fig. 1). MSI was not observed in benign melanocytic naevi. The primary malignant melanomas presented MSI in 4/25 cases, i.e. we observed an average of 16% MSI in malignant melanomas (Table 1). MSI was observed most frequently at the D10S89 and the D17S20 locus. In one tumour with Breslow thickness of 6.2 mm, MSI was seen at 2 loci; D10S89 and DSS346, respectively. Metastases showed the highest incidence of MSI (2/8). MSI was found in 1/12 of the tumours of <0.75 mm vertical thickness and in 3/13 of those >0.75 mm thick.

In addition to MSI, we observed LOH at chromosome 10 for both of the microsatellite markers tested. D10S89 showed LOH in 1/8 (12%) and D10S197 in 1/7 (14%) of the informative cases.

DISCUSSION

Two lines of evidence can be drawn from our data: 1) Our data confirm previous estimates of almost a quarter of all sporadic malignant melanomas being MSI in advanced stages. These findings require a more detailed evaluation of a possible prognostic consequence of a mismatched repair (MMR) deficit, since MSI status may have consequences for the response to therapy (3). In addition, there is preliminary evidence that aggressive chemotherapy makes it even more likely that MSI subclones, obviously resistant to chemotherapy, are selected (20, 21). Cell lines that were selected for resistance to chemotherapeutic agents such as etoposide and fotemustine exhibited a 2.0- to 2.5-fold increase in MSI rates, suggesting a link between MMR deficiency and drug resistance. This implies speculation that detection of a mismatch repair deficiency status in melanoma metastases may be of prognostic importance indicating an elevated risk for non-response to chemotherapeutic regimes. 2) The part that MSI/mismatch repair deficiency plays in the causal pathogenesis of sporadic melanocytic tumours is probably different from the intestinal system, as far as hereditary non-polyposis colorectal carcinomas are concerned and different from the conditions in Muir-Torre syndrome as well. In contrast to those conditions, in melanomas we have no evidence of an early onset of mismatch repair deficiency, for instance already in benign melanocytic naevi, comparable to actinic keratoses in Muir-Torre syndrome. Instead, the presence of a MSI status appears to be linked to the malignant phenotype and is more frequent for advanced stages, i.e. thick melanomas and metastases. Our results therefore support a progression-dependent accumulation of somatic mutations of microsatellites rather than MSI as an early causative event in melanomas.

Accordingly, the molecular genetics of melanoma MMR deficiency are different from the findings in hereditary non-polyposis colorectal carcinoma. Genes that are frequently mutated in colon cancer seem to retain integrity in malignant melanomas, even when a mutator phenotype can be observed. Variable levels of the mismatch repair gene hMSH2 were found to be expressed in melanoma cell lines, primary malignant melanomas, and melanoma metastases (22). Furthermore, the control-function of the p53 tumour suppressor protein on the hMSH2 expression turned out to be functional in malignant melanomas. Consequently, no mutations of hMSH2 or hMLH1 could be observed in MSI.
Table I. Overview of all specimens of primary malignant melanomas (pM) investigated and the occurrence of microsatellite instability (MSI)

<table>
<thead>
<tr>
<th>Primary melanomas</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Type</th>
<th>Clark’s level</th>
<th>Breslow’s index (mm)</th>
<th>Growth phase</th>
<th>Localization</th>
<th>MSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>pM1</td>
<td>m</td>
<td>52</td>
<td>SSM</td>
<td>III</td>
<td>0.8</td>
<td>H</td>
<td>trunk</td>
<td>–</td>
</tr>
<tr>
<td>pM2</td>
<td>m</td>
<td>40</td>
<td>SSM</td>
<td>IV</td>
<td>1.4</td>
<td>V</td>
<td>back</td>
<td>–</td>
</tr>
<tr>
<td>pM3</td>
<td>m</td>
<td>68</td>
<td>SSM</td>
<td>II</td>
<td>0.5</td>
<td>H</td>
<td>back</td>
<td>–</td>
</tr>
<tr>
<td>pM4</td>
<td>m</td>
<td>36</td>
<td>SSM</td>
<td>III</td>
<td>0.6</td>
<td>H</td>
<td>back</td>
<td>–</td>
</tr>
<tr>
<td>pM5</td>
<td>f</td>
<td>47</td>
<td>SSM</td>
<td>III</td>
<td>0.8</td>
<td>H</td>
<td>lower leg</td>
<td>D17S520</td>
</tr>
<tr>
<td>pM6</td>
<td>m</td>
<td>65</td>
<td>SSM</td>
<td>IV</td>
<td>1.8</td>
<td>V</td>
<td>trunk</td>
<td>–</td>
</tr>
<tr>
<td>pM7</td>
<td>m</td>
<td>75</td>
<td>SSM</td>
<td>IV</td>
<td>1.4</td>
<td>V</td>
<td>head</td>
<td>–</td>
</tr>
<tr>
<td>pM8</td>
<td>f</td>
<td>74</td>
<td>SSM</td>
<td>II</td>
<td>0.3</td>
<td>H</td>
<td>face</td>
<td>–</td>
</tr>
<tr>
<td>pM9</td>
<td>m</td>
<td>62</td>
<td>SSM</td>
<td>III</td>
<td>0.6</td>
<td>H</td>
<td>back</td>
<td>–</td>
</tr>
<tr>
<td>pM10</td>
<td>f</td>
<td>71</td>
<td>SSM</td>
<td>IV</td>
<td>0.9</td>
<td>V</td>
<td>shoulder</td>
<td>–</td>
</tr>
<tr>
<td>pM11</td>
<td>m</td>
<td>79</td>
<td>SSM</td>
<td>III</td>
<td>0.4</td>
<td>H</td>
<td>neck</td>
<td>–</td>
</tr>
<tr>
<td>pM12</td>
<td>f</td>
<td>61</td>
<td>SSM</td>
<td>IV</td>
<td>2.0</td>
<td>V</td>
<td>trunk</td>
<td>D10S89</td>
</tr>
<tr>
<td>pM13</td>
<td>m</td>
<td>43</td>
<td>SSM</td>
<td>III</td>
<td>0.8</td>
<td>H</td>
<td>trunk</td>
<td>–</td>
</tr>
<tr>
<td>pM14</td>
<td>m</td>
<td>73</td>
<td>SSM</td>
<td>III</td>
<td>0.6</td>
<td>H</td>
<td>back</td>
<td>–</td>
</tr>
<tr>
<td>pM15</td>
<td>m</td>
<td>54</td>
<td>SSM</td>
<td>III</td>
<td>1.1</td>
<td>H</td>
<td>shoulder</td>
<td>–</td>
</tr>
<tr>
<td>pM16</td>
<td>f</td>
<td>78</td>
<td>SSM</td>
<td>IV</td>
<td>1.9</td>
<td>V</td>
<td>back</td>
<td>–</td>
</tr>
<tr>
<td>pM17</td>
<td>m</td>
<td>58</td>
<td>SSM</td>
<td>II</td>
<td>0.5</td>
<td>H</td>
<td>forearm</td>
<td>–</td>
</tr>
<tr>
<td>pM18</td>
<td>m</td>
<td>47</td>
<td>SSM</td>
<td>II</td>
<td>0.6</td>
<td>H</td>
<td>trunk</td>
<td>–</td>
</tr>
<tr>
<td>pM19</td>
<td>f</td>
<td>42</td>
<td>SSM</td>
<td>II</td>
<td>0.2</td>
<td>H</td>
<td>forearm</td>
<td>–</td>
</tr>
<tr>
<td>pM20</td>
<td>f</td>
<td>66</td>
<td>SSM</td>
<td>III</td>
<td>0.4</td>
<td>V</td>
<td>forearm</td>
<td>–</td>
</tr>
<tr>
<td>pM21</td>
<td>f</td>
<td>57</td>
<td>NMM</td>
<td>IV</td>
<td>6.2</td>
<td>V</td>
<td>upper arm</td>
<td>D5S346 D10S89</td>
</tr>
<tr>
<td>pM22</td>
<td>m</td>
<td>68</td>
<td>ALM</td>
<td>II</td>
<td>0.2</td>
<td>H</td>
<td>praeputium</td>
<td>–</td>
</tr>
<tr>
<td>pM23</td>
<td>f</td>
<td>52</td>
<td>LMM</td>
<td>II</td>
<td>0.2</td>
<td>H</td>
<td>face</td>
<td>D17S520</td>
</tr>
<tr>
<td>pM24</td>
<td>f</td>
<td>35</td>
<td>SSM</td>
<td>IV</td>
<td>1.4</td>
<td>V</td>
<td>trunk</td>
<td>–</td>
</tr>
<tr>
<td>pM25</td>
<td>f</td>
<td>35</td>
<td>SSM</td>
<td>III</td>
<td>1.8</td>
<td>V</td>
<td>upper arm</td>
<td>–</td>
</tr>
</tbody>
</table>

H = horizontal, V = vertical.
SSM = superficial spreading malignant melanoma; NMM = nodular malignant melanoma; LMM = lentigo maligna melanoma, Clark’s level, Breslow’s index, vertical or horizontal growth and localization are summarized. ALM = acral lentiginous melanoma.

Further reason for turning to a larger scale of microsatellite analysis in melanomas comes from very recent studies indicating links between genome stabilization by administering non-steroidal antiphlogistic drugs. Rüschhoff et al. (3) investigated the effects of non-steroidal antiphlogistic drugs in mismatch repair deficient colorectal cancer cell lines and found that non-steroidal antiphlogistic drugs, such as aspirin or sulindac, induce a genetic selection for microsatellite stability in mismatch repair deficient cells. The use of non-steroidal antiphlogistic drugs may therefore provide an effective prophylactic therapy for hereditary non-polyposis colorectal cancer kindreds. One could speculate that non-steroidal antiphlogistic drugs might also be beneficial in keeping the genome stable in MMR-deficient malignant melanomas during aggressive chemotherapy. If using drugs like etoposide or fotemustine, non-steroidal antiphlogistic drugs could even help to avoid selection of MSI clones with resistance to the therapy applied.

REFERENCES

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